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USP's 2nd Bioassay Workshop

August 12-13, 2009
The Spalding Auditorium
USP Headquarters, Rockville, Maryland

Draft Agenda

Day 1 – Wednesday, August 12, 2009

8:00 a.m. Registration and Information, Continental Breakfast

8:30 a.m. **Opening and Welcome** — William Koch, USP

8:45 a.m. Workshop overview — Anthony Mire-Sluis, Amgen

Session I: USP Bioassay Guidance - Analysis

Chair: Robert Singer, Biometry Associates

9:00 a.m. The USP Bioassay Chapter Suite — Robert Singer, Biometry Associates

USP General Chapter <111>, Analysis of Biological Assays, was written in 1956, and remained unmodified for decades. In the new millennium the USP, working with empanelled statistical and biological scientists, has undertaken a major update of the chapter. <111> has been transformed into a suite of complementary chapters, with separate but inter-related focus on bioassay design, analysis, and validation. Current status of this work is presented.

9:30 a.m. From <111> to <1034> - What Has Changed? — Walter Hauck, USP

In 2008, USP published a proposed revision to General Chapter <111>, Design and Analysis of Biological Assays. Based on the recommendation of the responsible Advisory Panel and Expert Committee, that proposed revision will instead be a new General Chapter, <1034> Analysis of Biological Assays. The current proposal for <1034> incorporates responses to comments to what had been a <111> revision. This talk will summarize the status of <1034> and revisions to <111> and the major changes from 2008 that have been incorporated into <1034>.

10:00 a.m. European perspective — Alan Heath, NIBSC/HPA

10:30 a.m. **Break**

11:00 a.m. Parallelism Testing of Four-parameter Logistic Curves for Bioassays: A Case Study
Jun Kim & Harry Yang, MedImmune

11:30 a.m. <1034> Discussion with Panelists and Speakers

12:30 p.m. Lunch

Session II: USP Bioassay Guidance - Validation

Chair: Janice Callahan, Callahan Associates

1:15 p.m. <1033> Overview and Public Comments — Timothy Schofield, GSK

The USP chapter on Biological Assay Validation <1033> appeared in the March-April 2009 edition of Pharmacopeial Forum (Volume 35, No. 2). Chapter <1033> is the product of a joint effort amongst biologists, biochemists, and biostatisticians, and was offered for public comment. Modifications will be made which will help facilitate understanding and implementation of the bioassay validation principles delineated in the chapter. This talk will give an overview of the current chapter, as well as the significant comments which were received and will be acted upon. The enhancements which will appear in the revised chapter will be described and discussed.

1:45 p.m. Validation of Alternative Methods to Animal Testing - ECVAM's Principles — Marlies Halder, EVCAM

The European Centre for the Validation of Alternative Methods (European Commission Joint Research Centre, Institute for Health and Consumer Protection) main task is to validate methods which could replace animal tests. The presentation will summarise ECVAM's principle of validation with a specific focus on (immuno)biologicals

2:15 p.m. Bioassay Validation in Practice — Nancy Sajjadi

The ability of a company to bring a biopharmaceutical product to market is dependent on a myriad of factors including appropriate validation of any bioassays used to measure product activity and confirm its potency. While some companies have the resources and experience to develop and validate bioassays that meet with regulatory approval, others fail to do so. Avoidable mistakes, real life challenges, and the secrets for success in validating bioassays represent some of the lessons learned over many years of practice.

2:45 p.m. Panel Discussion

3:15 p.m. **Break**

Session III: USP Bioassay Guidance - Design

Chair: David Lansky, Precision Bioassay

3:30 p.m. <1032> Preview — Brian Peterson, Shire Inc.

Chapter <1032> represents an integral part of a multi-chapter effort intended to update the previous General Chapter <111> Design and Analysis of Biological Assays. Design of Biological Assays <1032> captures the fundamental principles essential to designing bioassay models that are amenable to rigorous product release and stability testing while also enabling the highest level of data interpretability. Successful bioassays are most often achieved through collaboration of the bioassay scientist with a properly trained statistician. This chapter addresses both expert audiences by providing guidance on good bioassay practices for the bioassay development scientist, with additional direction on how to approach the statistical design issues in bioassay for the practicing statistician. This talk will provide an overview of chapter scope, illuminate some issues the panel views as key in the chapter's development, and summarize the status of ongoing writing efforts.

4:00 p.m. Design of Bioassays – The Importance of Getting it Right — Rose Gaines-Das

Poor assay design wastes time and resources, and, most importantly, can result in misleading interpretations and conclusions. Valid interpretation and statistical analysis of experimental results depend on the assay design following sound principles. A first requirement is that experimental units are clearly identified and defined, and then they must be appropriately assigned to treatments. Failure to do this may invalidate interpretation of any statistical analysis. Implications for cell-based bioassays using 96-well micro-titre plates are considered as an example.

4:30 p.m. Bioassay Design Case Study — Pamela Mathis, Merck

With an increasing number of biologic programs moving through development, many companies are attempting to do more with limited resources and headcount. Other companies are trying to be as efficient and streamlined as possible in their processes. Cell based assay development can see benefits from approaches such as "staged validation" that can increase understanding of an assay earlier in the development cycle. This talk describes a 12 step process for developing cell based assays for use in potency determination and as stability indicating assays. The process incorporates Design of Experiments (DOE) to minimize work and maximize knowledge of the assay and the working space.

5:00 p.m. Round Table Discussion

5:30 p.m. **Reception**, Corridor of the Volunteers

Day 2 – Thursday, August 13, 2009

8:00 a.m. Continental Breakfast

Session IV: Vaccine Potency Assays

Chair: Tina Morris, USP

8:30 a.m. Regulatory Perspective — Phil Krause, FDA

9:00 a.m. Flu vaccines – Potency Assignment — Mark Galinski, MedImmune

9:30 a.m. Pandemic Flu – Special Considerations — Armen Donabedian, BARDA

BARDA seeks to leverage maturing technologies that can decrease the timeline of vaccine production. The preparation and calibration of influenza vaccine potency assay reagents constrains rapid delivery of vaccine supplies. Alternative assays, such as HPLC and LC-MS/MS, have been proposed as a substitute for and/or to augment the licensed assay. LC-MS/MS is being developed to reduce reagent delivery time by simplifying the assay calibration procedure. The longer term goal is to develop a potency assay that combines the measurement of antigenic or 'native' HA mass (e.g. using Abs, peptide mapping, functional binding assay or conformational analyses) with mass spectrometry and that relates back to established (e.g. NIST) standards.

10:00 a.m. **Break**

Session V: Monoclonal Antibodies – Relative Potency Assays

Chair: Michael Mulkerrin, Oncomed

10:30 a.m. Structural Integrity (STRINT) Assays Address a Key Quality by Design Need — Andy Goetze, Amgen

It has been stated that "Quality by Design (QbD) begins with the patient." What the patient minimally needs with respect to efficacy is a molecule that is both potent and exhibits sufficient circulating half-life. Monoclonal antibodies (mAbs) and peptibodies achieve a long circulating half life due to the presence of the Fc, which allows these molecules to be rescued from degradation via an FcRn-dependent mechanism. Without a functional Fc, such molecules would be expected to exhibit little efficacy in vivo due to rapid degradation.

We propose that "structural integrity" (STRINT) assays play a useful role in meeting QbD needs. These assays probe the simultaneous integrity of both ends of mAbs and peptibodies because they depend on both protein A (as a surrogate for FcRn) capture of the Fc and labeled ligand (or receptor) binding to the active site. STRINT assays are quickly developed, compatible with a high-throughput environment, and are typically highly sensitive to structural changes. For mAbs and peptibodies, STRINT assays may play a useful role in facilitating QbD during process and/or formulation development

11:00 a.m. Bioassays Evaluate Structure as Well As Function: Novel Probe of IgG2 Disulfide Isoforms — Richard Jerome, Pfizer

Differences in binding and cell based assays have been observed, sometimes when all other release specification limits and ranges were normal. These intriguing findings led to further investigations of the structure and function of monoclonal antibodies. A case study is provided where a difference in binding revealed information about structure independent of how the antibody interacts with its target. The impacts of these discoveries are enhanced applications for bioassays and appreciation of the value of closely examining data for trends and unusual phenomena.

11:30 a.m. Development of Surface Plasmon Resonance (SPR) Binding Assays for Lot Release and QC Laboratories — Ken Miller, MedImmune

Surface plasmon resonance (SPR) biosensors are routinely used for characterization of antibody-based therapeutics, and typically provide information about the binding kinetics (kon and koff) for the interaction between a therapeutic antibody and its target ligand. With the introduction of SPR biosensors specifically designed for GMP-regulated laboratories, the use of SPR technology in Lot Release and Quality Control (QC) laboratories has become more commonplace. This talk will focus on the development of binding assays for lot release and stability testing using SPR biosensors with particular attention given to experimental design considerations and data analysis.

12:00 noon The Roles and Requirements of the Potency Assay in the Development of Biopharmaceuticals — Carla Lankford, FDA

The potency assessment for biopharmaceuticals is a law requirement for licensure and it is expected the assay reflects the product's mechanism of action (MOA). Although developing a robust, sensitive, and MOA-reflective potency assay represents a significant challenge, it is expected that the type of potency assay proposed (i.e., cellular or acellular) is based on robust scientific data defining how the product interacts with and modifies its therapeutic target. An ideal potency assay should not only be reflective of the product's MOA, but also should be sensitive to product's structural modifications, stability indicating, and validated per ICH Q2 (R1). Establishing the appropriate assay specifications should be based on manufacturing, pre-clinical, and clinical experiences as appropriate for the product development phase.

12:30 p.m. **Lunch**

Session VI: Recombinant Therapeutics – Bioassays for Immunogenicity Testing

Chair: Tony Mire-Sluis

1:30 p.m. Regulatory Expectations — Susan Kirshner, FDA

Bioassays are an essential part of biological product development and potency measurements are required in several regulations, including the CFRs and ICH Q6B. Regulatory expectations exist around the need for potency assays - their nature (in vivo, in vitro, cell or binding based), their use (lot release, stability and/or characterization, immunogenicity testing) as well as their design, validation and analysis. Validation of cell based assays for use in immunogenicity testing focuses on different aspects of assay performance than validation for other uses. This talk will focus on aspects of assay validation that are both critical for immunogenicity testing, particularly specificity, selectivity and precision

2:00 p.m. Statistical Considerations for Immunogenicity Methods with Special Emphasis on NAb Bioassays — Viswanath Devanarayan, Abbott

Bioassays used for characterizing the neutralizing potential of anti-drug antibodies require careful statistical evaluation, some of which are quite different from the typical bioassays used in formulation studies. The standard curves tend to be significantly non-parallel to the dilution of the individual patient samples, and hence not usable for reporting the activity in the antibody concentration scale. So the assay signal is used directly for reporting the percent activity, and for the evaluation of important decision and assay performance characteristics such as the precision, sensitivity, cut point, and also titers when appropriate. In addition, the evaluation of cut points require careful consideration of sample selection strategy, distribution, inter-run differences, etc., that impact the type of cut point and the statistical approach used in the calculations

2:30 p.m. Current Technology for Advancing Bioassays Used in Immunogenicity Testing — An Song, Genentech

Bioassays used in immunogenicity testing face unique challenges which are different from bioassays used in potency testing. This talk will provide an overview of cell-based and binding-based technologies currently used in neutralizing antibody testing. Examples will also be given on technologies to potentially overcome neutralizing antibody assay specific challenges such as matrix interference and drug interference.

3:00 p.m. Designing Bioassays for Assessing Neutralizing Antibodies — Meena Subramanyam, Biogen Idec

Bioanalytical Assays and Bioassays form an integral part of the immunogenicity testing strategy and facilitate better understanding of the nature of the immune responses and characterization of the drug-specific antibodies. This talk will focus on the development of neutralizing bioassays that are robust and sensitive to detect anti-drug antibodies. The talk will specifically address assay design considerations for therapeutics with different modes of action (agonist Vs. antagonist) and highlight potential challenges in execution of these assays.

3:30 p.m. Immunogenicity Session Panel Discussion

4:00 p.m. Workshop Closure — Tina Morris, USP